ALKYLATION OF $tRNA^{Trp}$ IN A COMPLEX WITH TRYPTOPHANYL-tRNA SYNTHETASE

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1. Introduction

Specific interactions of tRNAs with cognate aminoacyl-tRNA synthetases have been studied by a number of ways (reviewed [1,2]). Among these approaches, chemical modification of the free tRNA as compared to the tRNA-synthetase complex is one of the most fruitful, since it shows directly the regions of tRNA molecule protected against chemical modification. For example, photo-crosslinking [3–5] and isotope-labeling [6] provide detailed information about the parts of the tRNA molecules involved in complex formation with synthetases. Using that approach, we have found that the phenylalanyl-tRNA synthetase from *Escherichia coli* shields the D-stem of the $tRNA^{Phe}$ from the guanosine-specific alkylating reagent MepURCl analogue of 2-chloroethylamine [7]. Alkylating reagents are especially useful for these purposes, since they, unlike other chemicals, modify the guanosine residues in the double-stranded as well as in the single-stranded regions of tRNA [7,8].

Here we describe the results of chemical modification by MepURCl of free $tRNA^{Trp}$ and its complex with beef tryptophanyl-tRNA synthetase. It is shown that the T-stem of the $tRNA^{Trp}$ is shielded by the bound enzyme.

Abbreviations: MepURCl, 2',3'-*O*-(4-(*N*-2-chloroethyl-*N*-methylamino)-benzylidene)-uridine-5'-methylphosphate

2. Materials and methods

RNase T2 was from Sankyo Co (Tokyo), RNase A from Calbiochem, DEAE-cellulose 23 SS (Serva). MepURCl was synthesized by Dr G. Karpova at the Novosibirsk Institute of Organic Chemistry as in [8]. Partially purified $tRNA^{Trp}$ was prepared by chromatography of the crude yeast tRNA on a benzoylated DEAE-cellulose column [9]. The enriched tRNA was aminoacylated with [^{3}H]tryptophan by pure tryptophanyl-tRNA synthetase and chromatographed on the benzoylated DEAE-cellulose column by elution with a concentration gradient of EtOH in 1 M NaCl. After deaminoacylation the $tRNA^{Trp}$ was finally purified by reversed-phase chromatography on a BPC-5 column [10].

Isolation of beef pancreas tryptophanyl-tRNA synthetase has been described [11], as have the methods of microcolumn chromatography [8,12]. Alkylation of the complex $tRNA^{Trp}$-tryptophanyl-tRNA synthetase was performed at 20°C in 0.025 M sodium cacodylate, pH 6.4, 0.005 M $MgCl_2$, 1×10^{-4} M EDTA. In this solution 1.5 A_{260} units of $tRNA^{Trp}$ were dissolved, and a 2-fold molar excess of enzyme was added (total vol. 60 μl). The concentration of MepURCl was 0.012 M. In the control experiments, addition of the enzyme to the reaction mixture was omitted. After the reaction, the $tRNA^{Trp}$-synthetase complex was isolated by gel-filtration through the Sephadex G-100 column (superfine, 0.3 × 40 cm)

equilibrated with the same buffer. The modified $tRNA^{Trp}$ and the enzyme were separated by chromatography on a DEAE-cellulose column (0.6 × 70 mm) with a linear gradient of NaCl (0–1.0 M, total vol. 600 μl). The isolated modified $tRNA^{Trp}$ was incubated for 40 min at 40°C in 0.2 M sodium acetate, pH 4.0, to split off the nucleotide moiety of the reagent [7,13]. Then the $tRNA^{Trp}$ was desalted by gel-filtration on a Sephadex G-25 column (0.2 × 15 cm) equilibrated with 0.02 M Tris–HCl, pH 7.7, and digested with RNase A. The oligonucleotides were analysed as described in [8]. For determination of their nucleotide composition the oligonucleotides were digested with RNase T2 and the nucleotides obtained were analysed by Dowex 1 microcolumn chromatography [7].

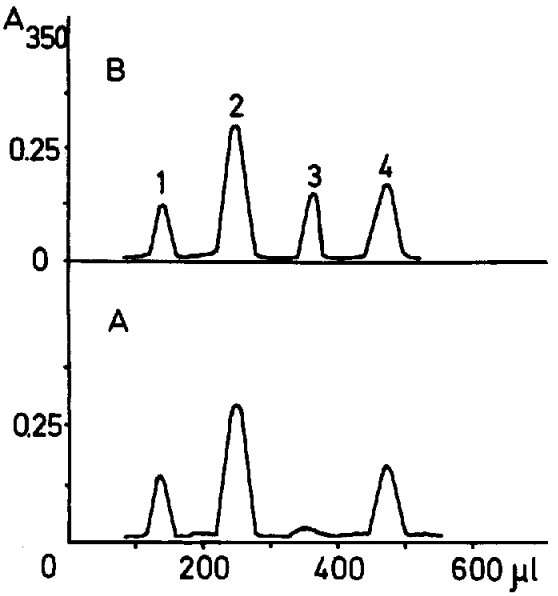


Fig.1. Chromatographic analysis of ribonuclease digests of modified $tRNA^{Trp}$. The average extent of modification of the $tRNA^{Trp}$ was 0.5 mol modified G/mol tRNA. (A) tRNA·synthetase complex. (B) tRNA. DEAE-cellulose column 0.6 × 30 mm, total amount of the $tRNA^{Trp}$ per analysis 1.0 A_{260} units. Concentration gradient of NaCl from 0.01–0.24 M in 0.02 M Tris–HCl (pH 8.2) and 7 M urea (total vol. 600 μl). Analysis of peak 3 after rechromatography at pH 3.7 showed it to contain the modified tetranucleotide A–G–G–Tp only.

3. Results and discussion

In our experiments the complex of $tRNA^{Trp}$ with tryptophanyl-tRNA synthetase was modified with the alkylating reagent MepURCl. The modification was performed at 20°C, pH 6.4, to the extent of no more than 0.5 mol reagent/mol tRNA on average. Therefore the patterns of alkylation obtained reflect reactivities of the guanosines in the native structure of $tRNA^{Trp}$ practically undisturbed by the modification.

In the control experiments the $tRNA^{Trp}$ was modified under the same conditions in the absence of the enzyme. After isolation from the reaction mixtures and acidic treatment, the samples of the modified $tRNA^{Trp}$ were digested with RNase A and the oligonucleotides were analysed by microcolumn chromatography on a DEAE-cellulose (fig.1). The modified oligonucleotides are easily detected spectrophotometrically due to A_{350} of the modified guanosines [12,13]. Comparison of the chromatographic patterns of the oligonucleotides of the ribonuclease digests of the $tRNA^{Trp}$ modified in the complex with the enzyme and in the free state shows that the former digest does not contain modified tetranucleotide (fig.1). Judging by chromatographic properties and nucleotide composition the modified oligonucleotide appears to be an alkylated tetranucleotide A–G–G–Tp. It originates from the T-stem of the $tRNA^{Trp}$ (fig.2). According to the general model of interaction of the tRNAs with the aminoacyl-tRNA synthetases proposed [14] the enzymes bind 'along and around the inside of the L-shaped tRNA structure'. The fact that the synthetases interact with the D- and the T-stem of tRNAs is rationalised by assuming that the enzymes may extend out and around the nucleic acid [14]. Sometimes the anticodon of the tRNAs is involved in the interaction, as with $tRNA^{Trp}$ from *E. coli* [15]. However, $tRNA^{Trp}$ does not contain guanosines in the anticodon loop, and therefore we could not observe this interaction. What we observed was a very pronounced protection of the oligonucleotide A–G–G–Tp in the T-stem of $tRNA^{Trp}$. All the other differences in the patterns of modification of $tRNA^{Trp}$ alkylated in the complex with the enzyme and in the free state were rather subtle. Earlier we have found that the phenylalanyl-tRNA synthetase from *E. coli* shields the D-stem of $tRNA^{Phe}$ from MepURCl [7]. The same sequence in the D-stem of $tRNA^{Trp}$ is not protected from

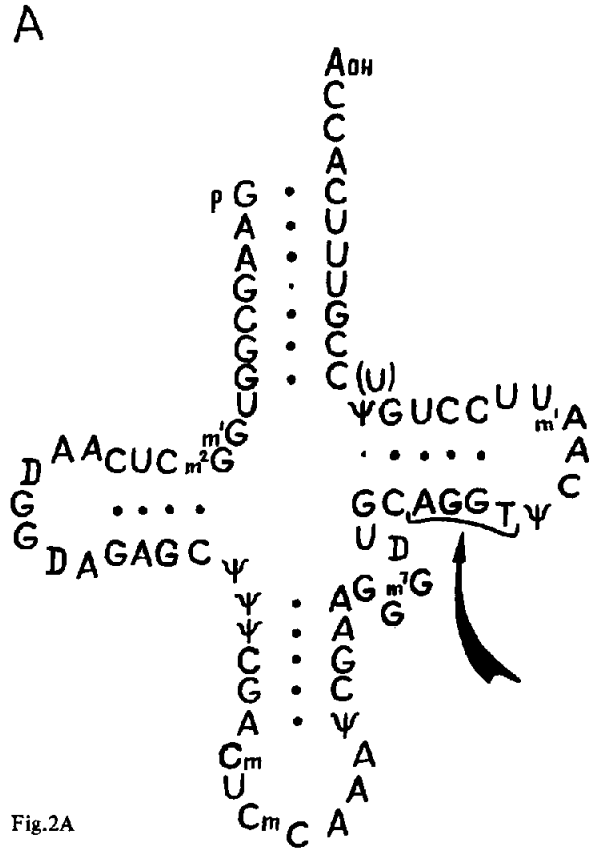


Fig.2. The clover-leaf structure (A) of yeast $tRNA^{Trp}$ [17] and the 3-dimensional structure (B) of yeast $tRNA^{Phe}$ [18]. The arrows show the position of the oligonucleotide protected by tryptophanyl-tRNA synthetase against alkylation.

MepURCl by the enzyme. Therefore it may be concluded that the D-stem of $tRNA^{Trp}$ is not involved in the interaction with the synthetase and that this interaction is not universal. It must be noted however that in both the cases, for the reagent to attack the N7 reactive centres of guanosines in the D- or the T-stems of tRNAs these centres should protrude between the stems. This section of the tRNA structure is rich in tertiary interactions and in sites for cation binding [16]. This part of tRNAs may be involved in recognition by the synthetases and the enzymes extend their segments between the D- and the T-stems of the tRNA structure to reach those parts of the tRNA structure responsible for the recognition.

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